# Water warming effects on the behavior of the African cichlid, *Julidochromis ornatus*

Undergraduate Honors Research Thesis

Presented in Partial Fulfillment of the Requirements for graduation "with Honors Research Distinction in Evolution and Ecology" in the undergraduate colleges of The Ohio State University

by

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April 2016

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**Abstract**

Human-induced rapid environmental change (HIREC) is threatening the world's flora and fauna. Aquatic ecosystems have proven to be particularly vulnerable to HIREC. Many of the world's largest lakes (e.g., Lake Tanganyika, East Africa) have seen rapid increases in water temperature. While previous research have shown that increased environmental temperature generally increases metabolic rates in ectotherms (e.g., fish), we know little about how this increase might affect their behavior (e.g., aggression). Since a heightened metabolism would raise the required energy for basic life functions, we hypothesized that aggression levels would be higher in individuals exposed to warmer temperatures relative to cooler ones. To test this hypothesis, we conducted a controlled laboratory experiment in which adult *Julidochromis ornatus* from Lake Tanganyika were exposed to low and high temperature treatments. As expected, average aggression levels were significantly higher in the warm (projected future) temperature treatment relative to the cooler (baseline) temperature. Despite this change in average aggression, individual aggression levels (measured before and at the end of the experiment) were positively correlated when all individuals were analyzed together. Collectively, these findings indicate that, while aggression in *J. ornatus* is plastic, individuals also have a personality that might constrain their response to HIREC. Ultimately, our findings suggest that continued warming in Lake Tanganyika, and many similar ecosystems, will change the behavior of its native fish fauna. As behavior appears critical to the reproductive success and social standing in many cichlid fishes, such changes might have major consequences for the ability of these fish species to persist in the face of continued HIREC.

## Introduction

Human-induced rapid environmental change (HIREC) has been pervasive in both terrestrial and aquatic ecosystems (Sih et al., 2011). HIREC encompasses habitat loss/fragmentation, spread of invasive species, human harvesting, pollution, and climate change, and has been attributed as a key reason behind altered species interactions, species declines, and range shifts (Sih et al., 2011). By causing rapid changes in an organism's environment, organisms exposed to HIREC may face selective pressures that have not been previously encountered in their evolutionary history (Sih et al., 2011). In turn, the resilience of organisms exposed to HIREC might be low. Because the thermal regime in tropical regions of the world has, historically, been more stable than in temperate regions, tropical species are expected to be more vulnerable to HIREC (e.g., climate change) than those species living in areas with historically higher environmental variation (e.g., temperate regions; Deutsch et al., 2008). As inhabitants of a tropical clime are rarely exposed to major environmental fluctuations, it can be presumed that the natural selective pressure for individuals to cope well with regular environmental fluctuations is weak. Such weak selection force would result in populations that are adapted to a stable, non-changing environment, likely increasing tropical species vulnerability to HIREC effects.

Understanding how tropical species react to HIREC is essential to preserving the unparalleled organismal diversity that is synonymous with the region. Many, if not most, of the effects of HIREC are experienced through the sensory system of the organisms (Sih et al., 2011). These changes in environmental cues could lead to differing results when organisms misevaluate formerly adaptive signals (Sih et al., 2011). These altered responses would likely be an important

factor in social animals that rely on behavior to help maintain sociality and hierarchy within groups (Maruska & Fernald, 2010). Consequently, learning how an organism's behavior changes under HIREC could help us better understand its effect on individual reproductive fitness and viability.

One particular area that has been experiencing multiple forms of HIREC is eastern Africa, which is home to several of the world's largest freshwater lakes, including Lake Tanganyika. Lake Tanganyika is one of the unique East African Rift Valley Lakes and is an important source of food, water, and trade for the people living along its shores (O'Reilley et al., 2003; Tierney et al., 2010). Because millions of people across nations depend on Lake Tanganyika for food and income, the ancient lake has been the victim of HIREC (O'Reilley et al., 2003; Tierney et al., 2010). As a result, Lake Tanganyika has experienced an unprecedented increase in temperature during recent decades, which appears to be the result of anthropogenic greenhouse gas emissions (Tierney et al., 2010). Climate warming also has been implicated in a reduction in primary production at the base of the food web in this ecosystem by altering the lake's thermal structure (O'Reilly et al., 2003).

Unfortunately, our understanding of how Lake Tanganyika's ecologically and economically important endemic fishes have and will continue to respond to continued HIREC is lacking. This information gap needs to be narrowed, given that the livelihood of local societies depends on the lake's richness, including its unique fish fauna. Lake Tanganyika supports a hugely diverse species assemblage that is composed of hundreds of fishes that are not found anywhere else in the world, primarily due to its large size, great depth, old age, and historically stable tropical climate (Tierney et al., 2010; O'Reilly et al., 2003). A better understanding of the

ability of this ecosystem to support its important fish assemblage in the face of HIREC could help drive conservation planning in the region.

While many questions exist with respect to how HIREC will impact Lake Tanganyika's endemic fishes, our understanding of their effect on individual behavior is especially lacking (Careau & Garland, 2012). This knowledge gap is especially important in tropical freshwater cichlids because individual behavior (e.g., aggression, boldness, exploration) can influence future fitness by potentially influencing mating success and ability to maintain social bonds. For example, many species of endemic cichlids in the East African Rift Valley lakes are pair-bonders for life, with mate-choice decisions by the female influenced by male behavior (Seehausen et al., 1998). In addition, individual behavior can influence future fitness by influencing adult survival. For instance, in environments with fish or human predators, being overly bold or aggressive has been shown to increase the risk of being preyed upon and harvested by commercial fishers respectively (Magurran & Seghers, 1991; Biro et al., 2009). Given the apparent importance of individual behavior to future fitness, studies that explore the impact of HIREC on individual behavioral traits are warranted.

In this thesis, we conducted a controlled laboratory experiment to quantify the impact of increased water temperature on aggression levels of the endemic Lake Tanganyika cichlid, *J. ornatus* (Golden Julie). We focused on temperature because of its great potential to alter fish behavior potentially through its effects on metabolic rate. A growing body of research with a variety of ectotherms has shown that the metabolism and behavior of individuals can be strongly correlated (Careau & Garland, 2012; Clarke and Johnston, 1999). For example, individuals with a faster metabolism have generally been found to be more aggressive and bolder than those with

a slower metabolism (Careau & Garland, 2012; Biro & Stamps, 2010). Although the relationship between metabolism and aggression is unclear, the alternative effects of temperature on metabolism provide evidence for possibly influencing behavior indirectly (Pörtner & Farrell, 2008; Pörtner & Peck, 2010). As our study population of *J. ornatus* showed a significant decrease in somatic growth rate and reproductive rate at the projected future temperature of Lake Tanganyika (29 ℃) relative to current baseline temperature (25 ℃), continued climate warming may exert substantial stress on this system's cichlid populations (Brodnik, 2015).

Because temperature and metabolism have generally been shown to be positively correlated in ectotherms, including *J. ornatus*, we predicted that individuals exposed to high temperature would be more aggressive or bolder than individuals exposed to a lower temperature (Campbell et al. 2008; Brodnik, 2015). This prediction, however, assumes that behavior is plastic, which might not be the case. While phenotypic plasticity in behavioral traits such as boldness and aggressiveness have been documented in ectotherms (e.g., Careau & Garland, 2012 and Biro & Stamps, 2010), other research has shown that the degree of plasticity in these behavioral traits can vary within and among species. In fact, with some individuals demonstrating a limited scope in behavioral responses (i.e., they exhibit a "personality", which is defined as repeatable behavioral responses that do not vary with the environmental context; Careau & Garland, 2012), a key question that could help better anticipate the effects of continued HIREC on Lake Tanganyika's endemic cichlids (e.g., *J. ornatus*) is whether individual aggressive behavior is fixed or exhibits a plastic response to environmental change. If the behavior of *J. ornatus* (and related cichlids) is shown to be more plastic than rigid in response to environmental changes that are physiologically stressful (e.g., higher temperature; O'Reilly et

al., 2003), individuals will be able to adjust their behavior to better compete for resources in a changing environment.

## Methods

### *Study system*

*J. ornatus* is a small-bodied, substrate-brooding cichlid endemic to Lake Tanganyika, which generally inhabits rocky shorelines in both the northern and southern sections of the lake (Awata & Kohda, 2004; Awata et al., 2005). *J. ornatus* commonly exhibits monogamy and sometimes polygamy or cooperative breeding (depending on the individual's size; Awata & Kohda, 2004; Awata et al., 2005; Awata et al., 2010). Fish were kept as monogamous pairs in our laboratory. As the organism is found commonly throughout Lake Tanganyika and is ecologically similar to many other small, substrate-brooding cichlid species, it sets itself as a good representative species for the diverse cichlid assemblage inhabiting the lake (Awata et al., 2005).

### *Experimental Set-up*

A total of 72 breeding pairs of adult *J. ornatus* that were two generations removed from Lake Tanganyika were formed. Each pair occupied a 57 L aquarium equipped with a breeding shelter. The aquaria were associated with four independent recirculating systems (n = 18 aquaria/system). The fish were kept under a fixed feeding regime with two pellets per adult fish provided daily throughout the whole experiment. Following an ~18 mos period in which water temperature in all recirculating systems was maintained at 25 ℃ (pre-treatment period), the temperature in each recirculating system was adjusted to 1 of 2 levels, 25 ℃ or 29 ℃, to mimic

the historically normal (low) and predicted future (high) temperature of Lake Tanganyika, respectively. The temperature treatment was implemented for 6 mos with one round of aggression assessment conducted before (pre-treatment) and after (post-treatment) implementation of the temperature exposure. This approach allowed us to have a maximum of 36 replicate breeding pairs in each temperature treatment (n = 2 recirculating systems per temperature; n = 18 aquaria per system). Because individuals died in all recirculating systems, both before and after implementation of treatment conditions, and we omitted "singles" from analyses (i.e., analyses only include aggression scores from individuals in intact breeding pairs to exclude the possible confounding effect of social status), samples sizes within each treatment were less than n = 36 per treatment and unbalanced between treatments (n = 19 to 36 individuals per treatment group).

*Behavioral Assessment*

Aggression trials were conducted at The Ohio State University's Aquatic Ecology Laboratory using 76 L aquaria, which were filled with water with a similar temperature, pH, nutrient concentration (nitrate, nitrate, and ammonia), and dissolved oxygen level as each test subject. Temperature and dissolved oxygen was recorded before and after each trial to ensure it did not change during the course of the 1 hour trials. Each test tank was divided into two sections: 1/6 of the tank designated as a holding area that was separated by a removable barrier from an area used to test exploratory behavior (additional behavioral tests were conducted as part of a broader study on the same system; A. Kua and A. McLaughlin, unpublished data). A high-definition (HD) video camera was mounted on a tripod to record the subject's behavior during the aggression trials. Aggressive behavior was assessed using methods developed by Shürch &

Heg (2010). This consisted of acclimating fish in the holding area for 10 min, then beginning the trial by replacing the barrier separating the aquarium's holding area from the rest of the tank with a mirror that faced the test subject. The behavior of each individual was recorded during the entire 10 min trial.

Individuals from each breeding pair were tested simultaneously in two identical aquaria (Figure 1), with individuals from each treatment tested in a semi-randomized order. More specifically, we only tested fish from the same temperature treatment on any given day (maximum of 4 fish per day), with the fish chosen for testing randomized within the two recirculating systems for that treatment. The order in which the two temperature treatments were selected for testing (on any given day) was randomized. Water changes (25%) were made after each trial, to minimize the influence of pheromones from individual influencing the behavior of individuals in subsequent tests. The methods followed established animal-use guidelines (IACUC protocol #2012A00000112).

*Data Analysis*

We used JWatcher™ version 1.0 to analyze the videos recorded for all behavioral trials. Multiple aggressive behaviors were measured, including both overtly aggressive behaviors (i.e., biting, ramming, tail slaps, and charging the reflection in the mirror) and more restrained aggressive acts towards the reflection (i.e., fin spreading, gill spreading, slow approaches, and side displays). These raw counts for each behavior were automatically summarized by JWatcher™ and then summed up for analysis into a total aggression value for each individual. All aggression tests were run, analyzed, and scored by a single observer, to maintain consistency when identifying aggressive behaviors.

We used a linear mixed model to explore how average aggression levels varied in response to temperature (low, high), the treatment period (pre-treatment, post-treatment), and their interaction, all of which were treated as fixed effects in our model. Individuals were included as a random effect in the model, and individual wet mass (nearest 1 g at the time that the second personality tests were measured) was included as a covariate. We used a least-squared (LS) means post-hoc comparison test to identify any significant differences between treatments. Mass of individuals in both treatments was compared via ANOVA to ensure similarity between treatments. The total aggression differences between male and female individuals were tested to enable us to simplify our model, excluding sex as an additional fixed effect in the linear mixed model (Welch Two Sample t-test: $T = 0.43$, $df = 102$, $P = 0.67$). Pearson's r correlation coefficient was used to test for behavioral consistency through time in individuals (pooled across all treatments), to investigate the possibility of personality in *J.ornatus*.

Total aggression count data were normal for the various treatment groups (Shapiro-Wilk's normality test: all $P \geq 0.16$). Likewise, all data met assumptions of homogeneous variances (Levene's test: all $P \geq 0.08$). All normality and homogeneity tests were conducted via STATISTICA 64 (ver. 12, Dell, Inc., Tulsa, OK), with the mixed modeling being conducted in R software environment (version 3.2.3) using the lme4, car, MuMIn, lsmeans, and lmerTest packages. Any test with a P-value $\leq 0.05$ was considered significant in reported analyses.

**Results**

Our linear mixed modeling analysis provided evidence to indicate that total aggression in our study population was plastic. While mass and time period were not significant in our model,

we found that temperature ($\chi = 10.80$ df = 1, $P < 0.005$) and the temperature x time interaction ($\chi = 7.91$, df = 1, $P < 0.005$) were significant (Table 1; Figure 2). Individual average wet mass increased significantly through time but did not differ with temperature when compared using a similar mixed model (Table 2). During the pre-treatment period, average individual aggression values did not differ between treatments with average total aggression values (pooled across all fish) in the pre-treatment period being 186 $\pm$ 7 (mean $\pm$1 standard error, SE; n = 65). Likewise, individual total aggression did not differ before and after implementation of the final temperature treatments in the low temperature (baseline) treatment. By contrast, average total aggression increased in individuals exposed to high temperature during the post-treatment period relative to the pre-treatment period (Figure 2). Average total aggression in the post-treatment period was 192 $\pm$8 (mean $\pm$SE; n = 49).

We also found evidence to indicate that aggression in *J. ornatus* is consistent. This notion was supported by the strong positive correlation found between the total aggression scores measured before and after exposure to the temperature treatments ($t = 2.8369$, df = 41, $P < 0.01$), albeit with spread among the data ($r = 0.41$, $P < 0.01$; Figure 3). Additionally, post-treatment average aggression levels (192.5), although not significant, were slightly lower when compared to pre-treatment (187.5) average aggression (Welch two sample t-test: T = -0.39, df = 84.8, P 0.70).

**Discussion**

Our study population of *J. ornatus* displayed some plasticity in aggressive behavior. This is evidenced by an increased average aggression in the high (future) temperature treatment

relative to the lower (baseline) temperature treatment. This finding supports our initial expectation, which was based on studies conducted with other ectotherms (Careau & Garland, 2012). These results are also intuitive, given the heightened metabolic rate observed in the same individuals held at the higher temperature (29 ℃) relative to the lower one (25 ℃) (Brodnik, 2015).

Because increased temperature in our study population of *J. ornatus* produced stark reductions in somatic growth and reproductive rates (Brodnik, 2015), the relationship between life-history and physiology may have an influence on individual behavior. With a heightened routine metabolic rate, individuals are expending more energy to carry out routine life functions, thus requiring greater energy (food) inputs. Because daily food rations were held constant throughout the experiment (just above maintenance levels; Brodnik, 2015), insufficient energy resources in the high temperature treatment may also have led to hunger becoming a secondary effect. Consequently, increased aggression may have stemmed from this food deficit. As food is likely to become a more limited resource in the predicted future environment of Lake Tanganyika, owing to reduced primary production (O'Reilly et al., 2003), we feel justified in our decision to hold feeding levels constant during the experiment. This decision to hold food constant between treatments is also justified, given that we do not know how exactly *J. ornatus* food sources are going to change in response to climate warming.

Even though high temperature caused a significant increase in individual aggression, we observed a general decrease in aggression in all treatments. This reduction in aggression may be due to a habituation effect, owing to individuals perhaps becoming less stressed in an increasingly familiar environment. Alternatively, the reduced aggression may be related to the

increase in body size that occurred between the first and second behavioral testing, which could have led to lower mass-specific metabolic rates.

This temperature-induced increase in aggression may affect individual fitness and viability. As *J.ornatus* breeds either in a monogamous pair or a hierarchical polygamous breeding group, heightened aggression probably would have a direct effect on individual mating success (Awata et al., 2005). Because the larger individual tends to be the more dominant individual in a breeding pair, the larger partner will likely use its size to exert dominance over its smaller mate and coerce it to spend more time carrying out parental duties (Awata et al., 2004). Even though defensive behaviors, which are shared by both parents, would likely improve due to a higher aggression level, it may also cause the more dominant partner to injure its mate, leading to a diminished ability for its mate to provide parental care to their young, reducing fitness as a result. While anecdotal, during the duration of our experiment, we observed multiple instances of mate aggression with the larger of the two exerting dominance over the lesser partner. This observed aggression (e.g., biting, charging, and fin raises) resulted both in injury and reduced feeding by the subordinate individual (A. Kua and S. Ludsin, unpublished data). In some cases, the subordinate partner died as a result of these aggressive acts.

The alternate form of reproductive behavior, i.e. cooperative breeding, would likely experience similar effects as a monogamous pair. Cooperative breeding in *J. ornatus* has been observed in two forms: 1) breeding pair with helpers; and 2) polygamous harems with helpers (Awata et al., 2005). Similar effects of elevated aggression could beleaguer breeding pairs with helpers, with the dominant partner in the breeding pair becoming more likely to injure and reduce the ability of its partner and helpers to effectively care for the brood. However, the same

increase in aggressive behavior would probably affect polygamous breeding groups in a more complex manner. Given the social hierarchy observed in polygamous harems, where size again is an indicator for social dominance (harem owner > breeders > helpers), harem owners may benefit from a higher level of aggression. Such an increase may allow individuals to more effectively control their harem, ensuring a higher level of fitness (Awata et al., 2005). The ability to maintain a larger harem under a more aggressive (dominant) individual may also lead to an overall higher reproductive success for the group, as a correlation between group size and group reproductive output has been documented (Awata et al., 2010) On the other hand, as we found an overall trend in increased individual aggression across our study population of *J. ornatus*, the same temperature effect may increase aggression in all members of a social group similarly. In this case, harem owners may face more frequent challenges from subordinates, disrupting the integrity of the social stability, possibly reducing mating success and fitness (Eldakar et al., 2009).

Unfortunately, we could not differentiate the social roles occupied by the cichlids in our test population, given that they were held in monogamous pairs. Hence, there was no way of knowing if there was a correlation between different social ranks and individual aggression. Assuming we have a somewhat equal number of individuals of various social ranks, the increase in aggression associated with the temperature increase seems to affect all individuals under that condition. This overall increase in aggression would likely mean that subordinate helpers or breeders may challenge their brood masters more frequently (Kochhann et al., 2015). As previous studies have found a relatively high level of genetic contribution of helpers to the next generation (helpers are mostly unrelated to dominant breeders), it would be interesting to explore

the effects of helper fitness under a higher environmental temperature where individuals are more aggressive.

Interestingly, we also found that aggression is not entirely plastic, as indicated by the significant positive correlation for individual total aggression scores before and after exposure to the temperature treatments. This correlation suggests that individuals that were initially more aggressive remained the more aggressive individuals in the population. This relationship suggests *J. ornatus* possess a personality, at least with respect to aggressive behavior. The possible existence of personality in *J. ornatus* could be strongly correlated with the group sociality which plays a strong role in the reproductive behavior of East African cichlids (Bergmüller & Taborsky, 2010). With a somewhat consistent behavior, individual differences in aggression may indicate an individual predisposition to certain social position. Consequently, more work needs to be conducted to investigate whether differences in behavioral types exist among individuals occupying different ranks in *J. ornatus* social groups. Behavioral type differences may have a more subtle effect and indicate the duration spent in each social role if such roles are due to life-history stages (Schürch & Heg, 2010). It would be interesting to uncover the basis for possible behavioral differences among harem owners, breeders, and helpers.

Additionally, inquiries into possible changes in other observable behavior in *J. ornatus* would allow us to obtain a clearer picture of how HIREC affects the behavioral phenotype of these tropical freshwater cichlids. Possible behaviors that would tie in appropriately with aggression would be exploration and boldness, as other studies performed on ectotherms have

found similar effects of temperature on these two traits besides aggression (Careau & Garland, 2012).

Given the possible fitness consequences that might stem from altered behavior in *J. ornatus*, understanding the relationship between metabolism and aggression would be the obvious next step to understanding the mechanism behind our observed results in *J. ornatus*. Correlating metabolism and aggression would allow us to better understand how a higher metabolism could impact organismal behavior (Ros et al., 2006; Kochhann et al., 2015). If a linkage exists between metabolic rate and aggression, it would allow us to more confidently identify HIREC as one of the key factors in decreasing individual fitness. Such research is vital to truly begin to understand whether ectotherms in tropical environs will be able to cope with climate change and perhaps other forms of HIREC.

It is also important to note that many HIREC effects occur concurrently (Sih et al., 2011). Thus, future studies should attempt to explore them in a multiple-stressor situation. For example, an increase in water temperature usually coincides with an increase in eutrophication events that lead to hypoxic (low dissolved oxygen) conditions (O' Reilly et al., 2003; Liikanen et al., 2002). As separate studies have found that coincident environmental changes may have confounding effects (Vanhoudt et al., 2012), investigations into how organisms would react to multiple stressors would allow us to more accurately predict HIREC effects.

**Limitations**

Our study had a few limitations that need to be taken into account. First, while each behavior observed was counted accurately with the aid of video recordings, the duration of said

behaviors was not analyzed. Even though all individuals performed similar aggressive behaviors, a considerable amount of variation existed in the duration of each observed behavior. For example, a biting behavior may only last for a fraction of a second for one individual but may take a few seconds for another. This creates an unequal assessment of the actual aggression that is shown by the individuals during the trials. Fortunately, this problem can be solved by reanalyzing the recorded videos via a more sophisticated method that accounts for the duration of each aggressive action performed by the test subject.

Additionally, the 25% water change protocol used to mitigate the effects of residual hormones left by the previous test subject has not been subjected to strong scrutiny for the method's true efficacy. Due to the unknown amount of hormones that were likely exuded by the fish during the behavioral trials, a 25% water change might not be able to flush out enough of the hormones from the water in the test tanks. However, given that the water used for the behavioral tests was collected from the home tank of the test subject (which is part of a multi-tank recirculating system), individuals are likely to be familiar with the hormones permeating the water as per when in its own tank. Even though the test subject may experience an increased stress level during behavioral trials, leaving behind a relatively high hormone residue, the potential cumulative effect should manifest equally in the results for each treatment (i.e., the bias would be similar between treatments). Hence, the effect of residual hormones, if any, would be cancelled out when different treatments are compared.

Another possible confounding factor is the cichlid social relationship chosen for the study. As mentioned earlier, the social relationship for all of the subjects was monogamous despite a variety of other social groups that form naturally in the wild (Awata et al., 2005). The

lack of knowledge of the underlying mechanism involved in the formation of different types of social groups in *J. ornatus*, combined with the increased social complexity of larger groups, means that we may have forced certain individuals into less than ideal social groups. If the various roles occupied by wild *J. ornatus* (e.g. harem owners, breeders, helpers, or monogamous partners) are fixed phenotypes, the effect of social role would have to be considered when trying to investigate individual behavioral changes (i.e., individuals cannot be assumed to be equal).

On a similar thread, changes to the monogamous relationship after treatment presented additional complications. Mortality among individuals in each system resulted in difference in sex ratios and social status. In particular, individuals from the high temperature treatment had fewer pairs at the time of the second behavioral trials. Our observation of a significant change in aggressive behavior could be skewed by the resulting imbalance of paired individuals that remained at the time of the second behavioral assessment. We tried to allay the problem by removing single subjects from our final analysis.

Lastly, as the test subjects were part of a larger study conducted to investigate the fitness effects of multiple stressors on *J. ornatus*, our aggression assessments were not the only tests undergone by the subjects. Individuals were put through respirometry trials throughout the treatment period and half of our population was subjected to relatively low levels of dissolved oxygen (DO) for a period of 3 mos. We tried to avoid conducting multiple assessments on individuals throughout the experiment, with only one type of test running at any given period of time. In addition, data collected from the respirometry tests suggests that the short-term low DO did not significantly impact the metabolism of the subjects, whereas fecundity data also did not

indicate any difference compared to normal DO treatments (A. Kua and R. Brodnik, unpubl. data).

**Acknowledgements**

We would like to thank The Ohio State University Office of Undergraduate Research and the Arts and Sciences College Honors Program for their generous support for this research, which came via a Summer Research Fellowship, a Research Scholar Award, and an Undergraduate Research Scholarship (all A. Kua). We also thank Sigma Xi for additional support for this research through a Grant-in-Aid of Research (to A. Kua). Lastly, a special thanks the many undergraduate volunteers (Kelli Ames, Erin Banaag, Taylor Brown, Joe Dillon, Steve Hadick, and Anissa Powell), St. Charles Preparatory High School volunteers (James Berry, Colin O'Connor), and Aquatic Ecology Laboratory technicians (Tyler Arnold, Jake Gilliland, Kim Winslow), graduate students (Reed Brodnik), and post-docs (Kristen DeVanna, Conor Keitzer) who helped with various aspects of our project.

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**Tables**

Table 1. Linear mixed model comparing total aggression scores with temperature, time, and temperature x time interactions as fixed effects, individual differences as random effects, and mass as a covariate.

| **Variable** | **$\chi^2$ value** | **Degrees of freedom** | **P-value** |
|---|---|---|---|
| Temperature | 10.80 | 1 | 0.001** |
| Time | 0.72 | 1 | 0.398 |
| Temperature x Time | 7.91 | 1 | 0.005** |
| Mass | 1.17 | 1 | 0.280 |

Table 2. Linear mixed model comparing the fixed effects of temperature, time, and temperature x time interactions and individual differences as random effects on mass differences.

| **Variable** | **$\chi^2$ value** | **Degrees of freedom** | **P-value** |
|---|---|---|---|
| Temperature | 0.000 | 1 | 0.998 |
| Time | 48.13 | 1 | < < 0.0001*** |
| Temperature x Time | 0.183 | 1 | 0.669 |

**Figures**

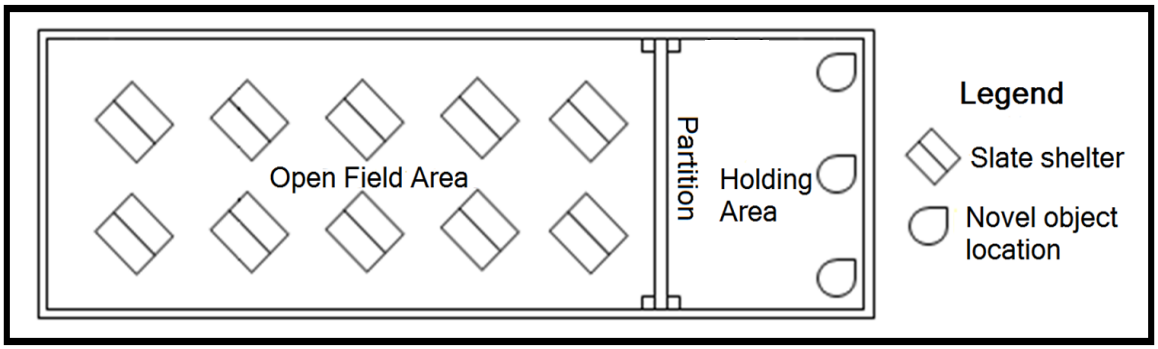


Figure 1. Tank setup for quantifying aggression, exploration, and boldness. Separate holding and open field areas were created for trials, with a mirrored partition placed facing the holding area during aggression trials. Aggression and boldness were assessed in the holding area; exploration was assessed in the open field area.

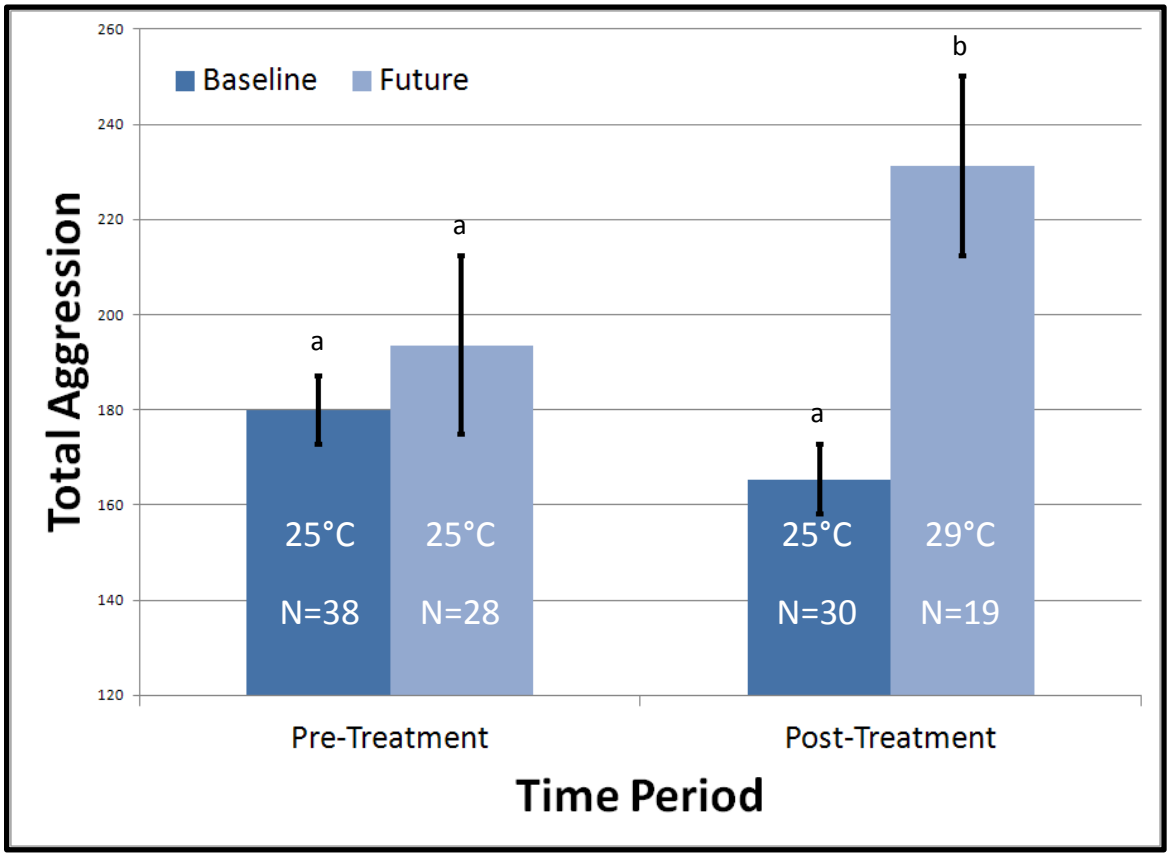


Figure 2. Mean (±1 SE) total aggression scores (sum of observed aggressive behaviors) before (pre-treatment) and after (post-treatment) implementation of temperature treatments. All pre-treatment and baseline temperature treatment = 25 ℃; Post-treatment future temperature = 29 ℃. Sample sizes (N) for each treatment are reported. Means with letters in common (above bars) do not differ (linear mixed model fit, LSmeans post-hoc comparison test: $P < 0.05$ for a vs. b).

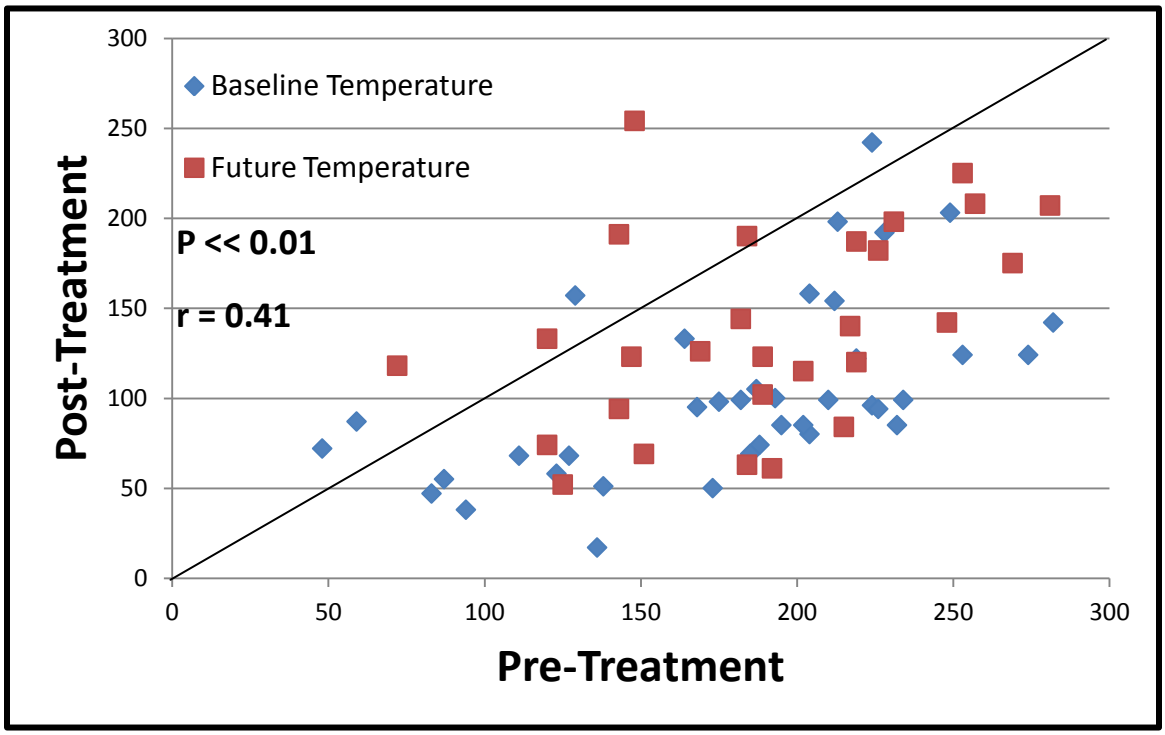


Figure 3. Pearson's r correlation between total aggression before (pre-treatment) and after (post-treatment) temperature treatments were implemented. Points represent individuals with red points indicating individuals in the high temperature treatment while blue points indicating individuals in the lower temperature treatments (N = 43). A one-to-one line was plotted for reference.